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13. ABSTRACT *(Maximum 200 Words)*

Information was obtained on the mechanistic sequence of events which occurs, and whether single or multiple regulatory points appear to be involved. Extensive information was obtained regarding the possible development of chemoprotective agents against structurally diverse environmental toxicants bsed on the use of antioxidants and free radical scavengers. In summary, the results will significantly contribute to our basic knowledge regarding the cascade of evennts which occurs following a toxic insult by structurally diverse xenobiotics. Finally, Information is being obtained on the ability of antioxidants/free radical scavengers and selected inhibitors to provide protection against these xenobiotics.

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FINAL TECHNICAL REPORT

September 1, 1997 – March 31, 1999

AFOSR Grant Number F49620-97-1-0016

COMMON PATHWAYS IN THE TOXICITY OF STRUCTURALLY DIVERSE XENOBIOTICS

by

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Submitted to

Air Force Office of Scientific Research
Bolling AFB, DC 20332-6448

I. OBJECTIVES

The mechanism of toxicity associated with exposure to structurally diverse environmental toxicants including heavy metals and polyhalogenated and/or polycyclic hydrocarbons may involve a common cascade of events which include the production of reactive oxygen species and an oxidative stress, glutathione depletion, altered calcium homeostasis, activation of the protein kinase C (PKC) system, enhanced release of tumor necrosis factor alpha (TNF-α), induction of stress/heat shock protein (HSP) 90, stimulation of oncogene expression and inhibition of tumor suppressor gene. Furthermore, the ultimate consequences include tissue damaging effects as lipid peroxidation, DNA single strand breaks, membrane damage with decreased membrane fluidity, and apoptosis (programmed cell death). A working hypothesis regarding this proposed cascade of events is presented in the Appendix. However, it is not clear whether structurally diverse xenobiotics activate this cascade of events by interaction of a single point or multiple regulatory points.

We have hypothesized that a common cascade of events occurs with respect to the toxicity of structurally diverse toxicants, and that structurally diverse xenobiotics can precipitate this series of events at diverse regulatory points and by diverse mechanisms. In order to address this hypothesis, both in vitro and in vivo studies are being conducted using four structurally unrelated environmental toxicants, namely, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), endrin, naphthalene and chromium (VI). These xenobiotics are being used to address the above questions, and determine the relationships between oxidative stress, DNA fragmentation, TNF-α, PKC, expression of bcl-2 gene, modulation of tumor suppressor gene p53 and programmed cell death (apoptosis).

Information is being obtained on the mechanistic sequence of events which occurs, and whether single or multiple regulatory points appear to be involved. Extensive information is also being obtained regarding the possible development of chemoprotective agents against structurally diverse environmental toxicants based on the use of antioxidants and free radical scavengers. In summary, the results will significantly contribute to our basic knowledge regarding the cascade of events which occurs following a toxic insult by structurally diverse xenobiotics. Finally, information is being obtained on the ability of antioxidants/free radical scavengers and selected inhibitors to provide protection against these xenobiotics.

II. STATUS OF EFFORT

Excellent progress has been made during the past year in meeting the specific objectives of this research project. Six manuscripts were published, and a seventh manuscript has been accepted for publication based on the results which have been completed. In addition, two manuscripts are in the process of being written. The results obtained have continued to provide significant insight into the molecular mechanisms involved in the production of toxicity by structurally diverse xenobiotics.

For more detailed information please refer to the Accomplishments/New Findings section of this report. Studies have been conducted with naphthalene, chromium, cadmium, TCDD, selected chemoprotectants and antioxidants.

Studies have been conducted in rats, mice, p53 deficient mice, and cell cultures including macrophage J774A.1 cells, human chronic myelogenic leukemic (CML K562) cells, promyelocytic leukemic HL-60 cells, and normal human peripheral blood mononuclear cells. Both single, oral, acute studies as well as daily, low dose chronic studies have been conducted with selected xenobiotics to which Air Force personnel are exposed through a variety of mechanisms. Most exposure to environmental toxicants involved chronic low dose exposure, and as a consequence, chronic low dose studies have been conducted with naphthalene, chromium, cadmium, and TCDD.

A number of new techniques involving laser scanning confocal microscopy, DNA laddering, and the use of p53 deficient mice have been used to address relevant questions. Taken together, the results support the basic hypothesis that the mechanism of toxicity of structurally diverse xenobiotics are at least in part induced through the production of the cascading series of events described above. The data also indicate that various xenobiotics influence different regulatory points within the cascade. Exciting and informative results are being obtained.

III. ACCOMPLISHMENTS/NEW FINDINGS

1. INDUCTION OF OXIDATIVE STRESS AND DNA DAMAGE BY CHRONIC ADMINISTRATION OF NAPHTHALENE TO FEMALE SPRAGUE-DAWLEY RATS.

Naphthalene is a bicyclic aromatic compound that is widely used in various domestic and commercial applications including lavatory scent disks, soil fumigants and moth balls. Little information is available regarding the mechanism of naphthalene toxicity. We have assessed the oral, low dose (0.05 LD_{50}) chronic effects of naphthalene (110 mg/kg/day p.o. in corn oil) for 120 consecutive days on lipid peroxidation and DNA fragmentation in the liver and brain tissues of female Sprague-Dawley rats. The animals were sacrificed on 0, 15, 30, 45, 60, 75, 90, 105 and 120 days of treatment. Maximum increases in hepatic and brain lipid peroxidation and DNA fragmentation were observed between 90 and 105 days of treatment. Following administration of naphthalene for 90 days, approximately 1.4- and 1.3-fold increases in lipid peroxidation were observed in the hepatic and brain tissues, respectively, while under the same conditions and time points 1.9- and 2.5-fold increases in hepatic and brain DNA fragmentation were observed, respectively. These results demonstrate that low dose chronic administration of naphthalene induces an oxidative stress resulting in tissue damaging effects that may contribute to the toxicity and carcinogenicity of naphthalene. This study has been published in Res. Comm. Mol. Path. Pharmacol. 101, 249-257 (1998)

2. NAPHTHALENE-INDUCED OXIDATIVE STRESS IN CULTURED J774A.1 MACROPHAGE CELLS.

In order to provide greater information regarding the mechanism associated with the ability of naphthalene to induce an oxidative stress, a series of *in vitro* experiments currently are in progress. Naphthalene is a bicyclic aromatic compound that is widely used in various domestic and commercial applications including lavatory scent disks, soil fumigants and moth balls. However, little information is available regarding the mechanism of naphthalene toxicity. In this project, we have assessed the concentration-dependent *in vitro* effects of naphthalene on increased lipid peroxidation, cytochrome c reduction, hydroxyl radical production, modulation of intracellular oxidized states by laser scanning confocal microscopy and DNA fragmentation in cultured macrophage J774A.1 cells. The cells were incubated with 0-500 μM concentrations of naphthalene for 0 and 24 hrs at 37°C. No significant changes were observed up to 100 μM. Lipid peroxidation increased by 1.5-, 2.4- and 2.9-fold at 100, 300 and 500 μM concentrations of naphthalene. Approximately 3.1-, 4.4- and 4.6-fold increases in cytochrome c reduction were observed at 300, 400 and 500 μM concentrations of naphthalene, respectively, demonstrating the production of superoxide anion, while under the same conditions approximately 3.2-, 4.2- and 4.9-fold increases in hydroxyl radical production were observed, respectively. Following incubation of these cells with 200 and 500 μM concentrations of naphthalene, 2.3- and 4.7-fold increases in average fluorescence intensity were observed, respectively, as compared to the untreated cells. Approximately 1.8-, 2.9- and 3.0-fold increases in DNA fragmentation were observed following incubation with 200, 400 and 500 μM concentrations of naphthalene, respectively. These results demonstrate that naphthalene may induce toxic manifestations by enhanced production of oxygen free radicals, resulting in lipid peroxidation, and DNA damage. These studies were published in Free Rad. Biol. Med. 25, 137-143 (1998).

3. CADMIUM (II) AND CHROMIUM (VI)-INDUCED OXIDATIVE STRESS AND CELL CYCLE MODULATION IN CULTURED HUMAN CHRONIC MYELOGENIC LEUKEMIC (CML K562) CELLS, PROMYELOCYTIC LEUKEMIC HL-60 CELLS AND NORMAL HUMAN PERITONEAL BLOOD MONONUCLEAR CELLS.

Both sodium dichromate [Cr(VI)] and cadmium chloride [Cd(II)] are cytotoxic and mutagenic. This *in vitro* study examined the toxic and apoptopic potentials of these two cations on cultured human chronic myelogenous leukemic (CML) K562 cells, promyelocytic leukemic HL-60 and normal human peripheral blood mononuclear cells. The cells were incubated with 0-100 μM concentrations of the two cations for 0, 24 or 48 hrs at 37°C. Cr(VI) and Cd(II) induced changes in intracellular oxidized states of cells which were detected using laser scanning confocal microscopy. Cell cycle modulation and apoptosis of the K562 cells by Cr(VI) and Cd(II) were determined by flow cytometry. Significant decreases in the G2/M phase were observed in the Cr(VI) and Cd(II) treated CML cells as compared to untreated cells. At 12.5 μM, Cr(VI) induced greater apoptosis in K562 cells as compared to Cd(II). In the K562 cells, 2.2- and 3.0-fold increases in

DNA fragmentation were observed following incubation with 12.5 and 25 μM Cr(VI), respectively, while 1.2- and 1.7-fold increases in DNA fragmentation were observed with Cd(II). Furthermore, approximately 2.7- and 4.9-fold increases in cytochrome c reduction were observed following incubation with 12.5 and 25 μM Cr(VI), respectively, while 1.6- and 3.3-fold increases in cytochrome c reduction were observed with Cd(II), demonstrating enhanced production of superoxide anion. Approximately 3.1 to 6-fold increases in hydroxyl radical production were observed following incubation of the K562 cells with these cations at 12.5 and 25 μM concentrations. These results were compared with promyelocytic leukemic HL-60 cells and normal human peripheral blood mononuclear cells. More pronounced effects were observed on K562 and HL-60 cells, while much lesser effects were observed on normal human peripheral blood mononuclear cells. The results demonstrate that both cations are toxic, producing oxidative tissue damage and apoptosis. Furthermore, more drastic effects were observed on K562 and HL-60 cells as compared to normal human peripheral blood mononuclear cells. The results of this study are in press in J. Biochem. Mol. Toxicol.

4. CADMIUM (II) AND CHROMIUM (VI)-INDUCED OXIDATIVE STRESS AND PROGRAMMED CELL DEATH IN CULTURED J774A.1 MACROPHAGE CELLS.

Sodium dichromate (VI) and cadmium chloride (II) are known to induce cytotoxicity and mutagenesis. This in vitro study was designed to focus upon the toxic and apoptopic potential of these cations on cultured J774A.1 cells. These cells were incubated with 0.20, 0.40, and 0.60 μM concentrations of these cations for 0, 24 or 48 hrs at 37°C. Chromium or cadmium-induced changes in cell morphology were detected using phase contrast microscopy. The overall intracellular oxidized states of cells were measured at an excitation wavelength of 513 nm by laser scanning confocal microscopy using 2,7-dichlorofluorescein diacetate (DCFD) as the probe. Concentration dependent increases in fluorescence intensity were observed. Signal was quantitated by integrating fluorescence over a user defined area cell number. Approximately 3.6-8.2 fold increase in fluorescence intensity were observed following treatment with chromium or cadmium ions. Concentration- and time-dependent influences of chromium and cadmium ions on succinate dehydrogenase, a marker of mitochondrial electron transport chain, were determined using the MTT assay. Significant increases in enzyme activity were observed with both these ions at 0.40 and 0.60 μM concentrations at 24 hrs, while a significant decrease in enzyme activity was observed at 48 hrs. Chromium produced a more pronounced effect as compared to cadmium ions. Fragmentation of nuclear DNA is a biochemical hallmark of programmed cell death (apoptosis), which was assessed using TUNNEL (TdT-mediated dUTP-biotin nick end labelling) method employing fluorescent microscopy. Concentration- and time-dependent induction of apoptosis was observed with both cations. Chromium was shown to induce more toxic effects at lower concentrations than cadmium ions. These results clearly indicate that both cations are toxic, producing oxidative tissue damage and apoptosis. The manuscript was published in In Vitro Mol. Toxicol. 11, 171-181 (1998).

5. COMPARATIVE INDUCTION OF OXIDATIVE STRESS IN CULTURED MACROPHAGE J774A.1 CELLS BY CHROMIUM PICOLINATE AND CHROMIUM NICOTINATE.

The concentration-dependent effects of chromium picolinate (Cr Pic) and chromium nicotinate (Cr Nic) were assessed on the enhanced production of superoxide anion and hydroxyl radicals, lipid peroxidation and DNA fragmentation in cultured macrophage J774A.1 cells. The cells were incubated with 0-50 μg/ml concentrations of these chromium (III) salts for 0 and 24 hrs at 37°C. Concentration-dependent effects were observed. Lipid peroxidation increased by 1.3 - 1.5-fold following treatment of these cells with Cr Pic and 1.2 - 1.8-fold at these same concentrations of Cr Nic. Increases of 1.0 - 1.5-fold occurred in the production of superoxide anion as determined by cytochrome c reduction following treatment of these cells with Cr Pic while with these same concentrations and conditions only 1.1 - 1.2-fold increases were observed following treatment with Cr Nic. Approximately 1.2 - 1.5-fold increases in hydroxyl radical production were observed following treatment of these macrophage cells with increasing concentrations of Cr Pic and Cr Nic. Incubation of these cells with 30 - 50 μg/ml concentrations of Cr Pic produced 1.2 - 1.6-fold increases in DNA fragmentation, while under these same conditions with Cr Nic 1.2 - 1.3-fold increases occurred. No significant loss in cell viability was observed with either chromium salt. These results demonstrate that incubation of macrophage J774A.1 cells with these chromium salts induces low levels of oxidative stress as demonstrated by the biochemical assay techniques employed in this study. These studies were published in Res. Comm. Mol. Path. Pharmacol. 97, 335-346 (1998).

6. COMPARATIVE EFFECTS OF TCDD, ENDRIN, NAPHTHALENE AND CHROMIUM (VI) ON OXIDATIVE STRESS AND TISSUE DAMAGE IN THE LIVER AND BRAIN TISSUES OF C57BL/6J MICE.

The mechanism of toxicity of structurally diverse environmental toxicants including heavy metals and polyhalogenated and polycyclic hydrocarbons may involve a common cascade of events which entails an oxidative stress and production of reactive oxygen species. We have determined the comparative effects of single 0.10 and 0.50 LD_{50} doses of TCDD, endrin (EN), naphthalene (NAP) and sodium dichromate (CrVI) on lipid peroxidation (LP), DNA fragmentation (DF) and enhanced production of superoxide anion (cytochrome c reduction; CCR) in the liver and brain tissues of mice at 0, 12, 24, 48, 72 and 96 hr after treatment. Dose- and time-dependent effects were observed with all four xenobiotics. At a 0.50 LD_{50} dose TCDD, EN, NAP and CrVI, maximum increases in CCR were approx. 5.8-, 4.9-, 7.3- and 6.0-fold in hepatic tissues. TCDD showed an increasing effect through 96 hr. EN and NAP demonstrated a maximum effect at 12 hr, while CrVI demonstrated a maximum effect at 48 hr. With respect to LP, at a 0.50 LD_{50} dose both EN and CrVI induced the maximum effect at 48 hr of treatment, while NAP demonstrated the maximum effect at 12 hr. TCDD demonstrated a continued

effect through 96 hr of treatment. At a 0.50 LD_{50} dose TCDD, EN, NAP and CrVI produced maximum increases in hepatic LP of approx. 3.5-, 3.1-, 3.5- and 3.3-fold in hepatic tissues, respectively. Similar results were obtained in hepatic and brain DF at 0.50 LD_{50} doses. NAP demonstrated the maximum effect at 24 hr while both EN and CrVI showed the maximum DF at 48 hr. Lesser effects were observed with 0.10 LD_{50} doses of these xenobiotics. Thus, these diverse xenobiotics induce dose- and time-dependent oxidative stress and tissue damage in the liver and brain tissues of mice. An abstract was presented at the annual meeting of the Society of Toxicology, 1998. A manuscript has been submitted to Free Rad. Biol. Med.

7. ROLE OF TUMOR SUPPRESSOR GENES IN THE TOXICITY OF TCDD, ENDRIN, NAPHTHALENE AND CHROMIUM (VI).

It has been postulated that tumor suppressor genes are involved in the cascade of events leading to the toxicity of diverse xenobiotics. Therefore, we are assessing the comparative effects of 0.01 LD_{50}, 0.10 LD_{50} and 0.50 LD_{50} doses of TCDD, endrin, naphthalene and chromium (VI) in p53 deficient mice in order to determine the role of p53 in the toxic manifestations produced by these diverse xenobiotics. In general, p53 deficient mice are more susceptible to all four xenobiotics. Specifically, naphthalene and chromium induced greatest toxicity at the 0.50 LD_{50} dose in liver of the mice, while naphthalene and endrin induced the greatest effect in brain tissue. At a 0.10 LD_{50} dose, TCDD, naphthalene and endrin induced significant effects in both liver and brain while at the lowest dose (0.01 LD_{50}), toxic effects were produced by TCDD, chromium and naphthalene in the liver while TCDD and chromium induced some toxic effects in the brain tissues. These results have been combined with the results described above under Section 6, and a large manuscript has been submitted to Free Rad. Biol. Med.

We shall conduct similar studies assessing the role of bcl-2 in mice in the future.

8. PROTECTIVE EFFECT OF MELATONIN ON NAPHTHALENE, TCDD, ENDRIN AND CHROMIUM (VI) INDUCED OXIDATIVE STRESS AND DNA DAMAGE IN CULTURED MACROPHAGE J774A.1 CELLS.

Naphthalene (NAP) is a bicyclic aromatic compound that is widely used in various domestic and commercial applications including lavatory scent disks, soil fumigants and moth balls. Previous studies in our laboratory have demonstrated enhanced production of reactive oxygen species, lipid peroxidation (LP) and DNA fragmentation in both *in vitro* and *in vivo* models. In this study, we have investigated the protective ability of 0.50 and 1 mM melatonin (N-acetyl-5-methoxytryptamine) against NAP-induced oxidative stress and DNA damage in cultured macrophage J774A.1 cells. No significant changes were observed with concentrations of NAP up to 100 μM. Approx. 2.0-, 3.1- and 4.6-fold increases in cytochrome c reduction were observed at 200, 300 and 500 μM concentrations of NAP, demonstrating the production of superoxide anion. At 24 hrs, lipid peroxidation increased by 1.8-, 2.4- and 2.9-fold following treatment of these cells with 200, 300 and 500 μM concentrations of NAP, respectively, while 1.8 to 3.0-fold

increases in DNA fragmentation were observed at these same concentrations. Pretreatment of these cultured cells with 1 mM melatonin provided 30% and 25% decreases in superoxide anion and lipid peroxidation production, respectively, in cells treated with 300 μM NAP. Similar results were observed for DNA fragmentation. These data demonstrate that melatonin can significantly protect against NAP-induced oxidative stress and DNA damage in cultured cells. An abstract was presented at the annual meeting of the Society of Toxicology, 1998.

9. MODULATION OF TCDD-INDUCED FETOTOXICITY AND OXIDATIVE STRESS IN EMBRYONIC AND PLACENTAL TISSUES OF C57BL/6J MICE BY VITAMIN E SUCCINATE AND ELLAGIC ACID.

The ability of vitamin E succinate and ellagic acid to modulate 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced developmental toxicity and oxidative damage in embryonic/fetal and placental tissues was studied in C57BL/6J mice. Vitamin E succinate (100 mg/kg per day) and ellagic acid (6 mg/kg per day) were administered by gavage to groups of pregnant mice on days 10, 11 and 12 of gestation and 40 mg vitamin E succinate/kg or 3 mg ellagic acid/kg on day 13 of gestation. A number of animals from the vitamin E succinate and ellagic acid treated groups also received 30 μg TCDD/kg on day 12 of gestation, 2 h prior to vitamin E succinate or ellagic acid treatment. Groups of treated animals were terminated on day 14 of gestation, and the biomarkers of oxidative stress, including superoxide anion production and the induction of lipid peroxidation and DNA-single strand breaks (SSB), were determined in whole embryonic and placental tissues homogenates. Groups of treated animals were also killed on day 18 of gestation for investigation of the fetotoxic and teratogenic effects as well as effects on the placentae. Vitamin E succinate and ellagic acid significantly decreased TCDD-induced fetal growth retardation fetal death and placental weight reduction, with no significant ameliorating effects on TCDD-induced malformations including cleft palate and hydronephrosis. Vitamin E succinate treatment resulted in decreases of 77-88%, 70-87%, and 21-47% in the production of superoxide anion, lipid peroxidation and DNA-SSB, respectively, in embryonic and placental tissues, while ellagic acid caused 47-98%, 79-93%, and 37-53% decreases, respectively, in these parameters. These results indicate that TCDD-induced fetal death and fetal and placental weight reductions in C57BL/6J mice may be due to oxidative damage induced by TCDD, and ellagic acid and vitamin E succinate provide protection against those effects. Ellagic acid provided better protection than vitamin E succinate against TCDD-induced fetal growth retardation and increases in lipid peroxidation in embryonic and placental tissues. This study was published in Toxicology 124, 27-37 (1997).

10. INDUCTION OF OXIDATIVE STRESS IN BRAIN TISSUES OF MICE AFTER SUBCHRONIC EXPOSURE TO TCDD.

The ability of single doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to induce oxidative stress in hepatic and some extrahepatic tissues of animals is well documented.

However, no previous study has examined the ability of TCDD to induce oxidative stress and tissue damage in brain *in vivo*. In this study the ability of TCDD to induce oxidative stress in brain tissues of mice was studied after subchronic exposures. Groups of female B6C3F1 mice were treated orally with TCDD (0, 0.45, 1.5, 15, and 150 ng/kg/day) for 13 weeks, 5 days/week. The animals were euthanized 3 days after the last treatment and brain tissues were collected. Biomarkers of oxidative stress including production of superoxide anion, lipid peroxidation, and DNA-single strand breaks (SSB) were determined. TCDD treatment resulted in significant and dose-dependent increases in the production of superoxide anion as assessed by reduction of cytochrome c. Significant increases were also observed in lipid peroxidation and DNA-SSB in those tissues, as assessed by the presence of thiobarbituric acid-reactive substances and the alkaline elution technique, respectively. These results clearly indicate that subchronic exposure to low doses of TCDD can induce oxidative tissue damage in brain tissues which may at least in part play a role in the effects of TCDD on the central nervous system. This study was published in Toxicol. Sci. 42, 23-27 (1998).

IV. PERSONNEL SUPPORTED

Dr. Sidney J. Stohs - Principal Investigator
Dr. Debasis Bagchi - Co-investigator
Dr. Ezdihar Hassoun - Co-investigator
Dr. Manashi Bagchi - Co-investigator
Dr. Naser Z. Alsharif - Collaborating Investigator
Dr. Linda S. Birnbaum - Collaborating Investigator
Dr. Michael DeVito - Collaborating Investigator
Dr. Angelique Van Birgelen - Collaborating Investigator
Dr. Siddhartha D. Ray - Collaborating Investigator
Dr. S.S. Joshi - Collaborating Investigator
Dr. Charles A. Kuszynski - Collaborating Investigator
Dr. Sean Newton - Collaborating Investigator
Mr. E.J. Benner - Technician
Ms. Lin Tang - Technician
Ms. Jaya Balmoori - Technician
Ms. Xumein Ye - Technician
Mr. Keith Mele - Student
Mr. Phillip Vuchetich - Student
Mr. A.C. Walter - Student
Mr. Minh X. Tran - Student
Mr. Amit Garg - Student
Mr. Casey B. Williams - Student
Ms. Dipanjali Bagchi - Student
Ms. Nicole A. Carryl - Student

V. PUBLICATIONS

1. D. Bagchi, M. Bagchi, J. Balmoori, P.J. Vuchetich and S.J. Stohs. Induction of oxidative stress and DNA damage by chronic administration of naphthalene to rats. Res. Comm. Mol. Path. Pharmacol. 101, 249-257 (1998).

2. D. Bagchi, S.S. Joshi, M. Bagchi, J. Balmoori, E.J. Benner, C.A. Kuszynski and S.J. Stohs. Cadmium and chromium induced oxidative stress, DNA damage and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL-60 cells and normal human peripheral blood mononuclear cells. J. Biochem. Mol. Toxicol. (in press).

3. E.A. Hassoun, A.C. Walter, N.Z. Alsharif and S.J. Stohs. Modulation of TCDD-induced fetotoxicity and oxidative stress in embryonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid. Toxicology 114, 27-37 (1997).

4. M. Bagchi, M. Bagchi, J. Balmoori, X. Ye and S.J. Stohs. Comparative induction of oxidative stress in cultured J774A.1 macrophage cells by chromium picolinate and chromium nicotinate. Res. Commun. Mol. Path. Pharmacol. 97, 335-346 (1997).

5. M. Bagchi, D. Bagchi, J. Balmoori, X. Ye and S.J. Stohs. Naphthalene-induced oxidative stress and DNA damage in cultured macrophage J774A.1 cells. Free Rad. Biol. Med. 25, 137-143 (1998).

6. E.A. Hassoun, S.C. Wilt, M.J. DeVito, A. Van Birgelen, N.Z. Alsharif, L.S. Birnbaum and S.J. Stohs. Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Tox. Sci. 42, 23-27 (1998).

7. D. Bagchi, M.X. Tran, S. Newton, M. Bagchi, S.D. Ray, C.A. Kuszynski and S.J. Stohs. Chromium and cadmium induced oxidative stress and apoptosis in cultured J774A.1 macrophage cells. In Vitro Mol. Toxicol. 11, 171-181 (1998).

VI. INTERACTIONS/TRANSITIONS

A. PARTICIPATION/PRESENTATIONS AT MEETINGS/CONFERENCES

1. Comparative induction of oxidative stress in cultured macroophage J774A.1 cells by chromium picolinate and chromium nicotinate. D. Bagchi, M. Bagchi, J. Balmoori, X. Ye and S.J. Stohs. Society of Toxicology, 1998. Seattle, WA.

2. Comparative effects of TCDD, endrin, naphthalene and chromium (VI) on oxidative stress and tissue damage in the liver and brain tissues of C57BL/6J mice. S.J. Stohs, C.B. Williams, A. Garg, J. Balmoori, X. Ye, K. Mele, N.A. Carryl, M. Bagchi and D. Bagchi. Society of Toxicology, 1998. Seattle, WA. Also presented in the

Midwest Student Medical Research Forum XXVIII, 1998. Omaha, NE.

3. Protective effect of melatonin on naphthalene-induced oxidative stress and Dna damage in cultured macrophage J774A.1 cells. J. Balmoori, M. Bagchi, X. Ye, D.J. Bagchi, D. Bagchi and S.J. Stohs. Society of Toxicology, 1998. Seattle, WA.

B. CONSULTATIVE AND ADVISORY FUNCTIONS

Dr. S.J. Stohs served as a consultant to the University of Athens and the Greek government on the role of metal ions in toxicity, 1997.

Dr. D. Bagchi chaired and conducted a session entitled "Free radicals, apoptosis and human health: Role of antioxidants and micronutrients" at the Annual Meeting of the American College of Nutrition, Albuquerque, NM, October, 1998.

Dr. S.J. Stohs has been appointed to the Medical/Scientific Advisory Board of AdvoCare International, Dallas, TX.

C. TRANSITIONS

1. Determination of lipid metabolites (including malondialdehyde, formaldehyde, acetaldehyde and acetone) by HPLC and GC-MS -- transferred to:

   a. Dr. Ezdihar Hassoun, Department of Pharmacology, University of Toledo, Toledo, OH 43606.

   b. Professor Dipak K. Das, Department of Surgery, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030. Three papers have been published using these techniques.

   c. Professor Thomas E. Adrian, Director of Cancer Research, Creighton University School of Medicine, Omaha, NE 68178.

   d. Professor Parviz M. Pour, Department of Pathology and Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE 68198.

2. Determination of DNA fragmentation following oxidative insult -- transferred to:

   a. Professor Dipak K. Das, Department of Surgery, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030.

   b. Dr. Ezdihar Hassoun, Department of Pharmacology, College of Pharmacy, University of Toledo, Toledo, OH 43606.

VII. NEW DISCOVERIES, INVENTIONS OR PATENT DISCLOSURES

None - The report of Inventions and Subcontracts is enclosed.

VIII. HONORS/AWARDS

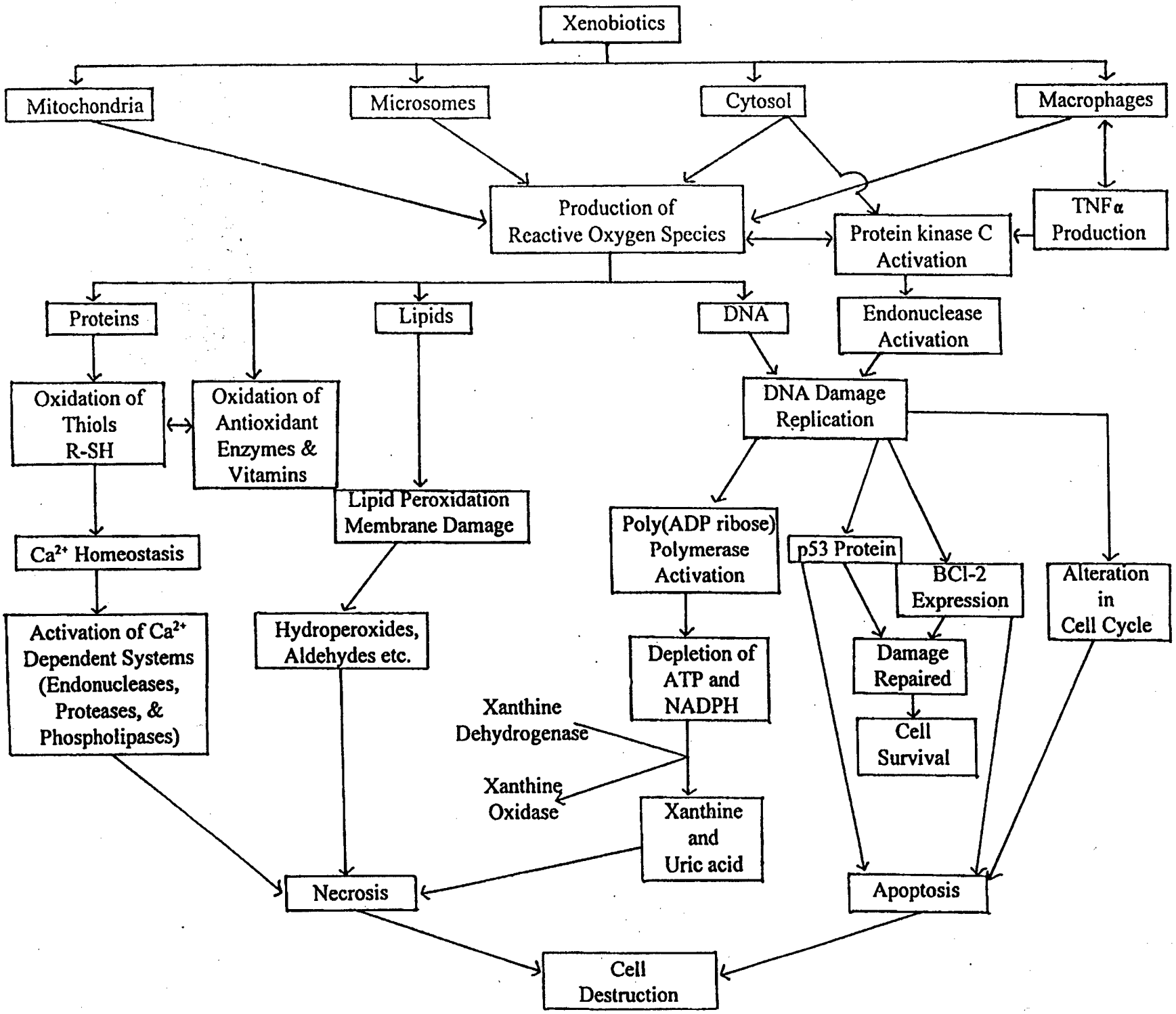
Dr. S.J. Stohs was appointed to a Greek Government Advisory Board on Tobacco Toxicity.

Dr. Debasis Bagchi was asked to chair and organize a symposium entitled "Free Radicals, DNA Damage, Apoptosis and Human Health: Role of Antioxidants and Micronutrients" in the Annual Meeting of the American College of Nutrition, Albuquerque, NM, 1998.

Dr. S.J. Stohs was elected a Fellow of the American College of Nutrition, 1999.

APPENDIX #1

WORKING HYPOTHESIS REGARDING THE CASCADE OF EVENTS ASSOCIATED WITH THE MECHANISMS OF TOXICITY OF STRUCTURALLY DIVERSE ENVIRONMENTAL TOXICANTS


Xenobiotics
Mitochondria
Microsomes
Cytosol
Macrophages
Production of Reactive Oxygen Species
Protein kinase C Activation
TNFα Production
Proteins
Lipids
DNA
Endonuclease Activation
Oxidation of Thiols R-SH
Oxidation of Antioxidant Enzymes & Vitamins
DNA Damage Replication
Lipid Peroxidation Membrane Damage
Poly(ADP ribose) Polymerase Activation
p53 Protein
BCl-2 Expression
Alteration in Cell Cycle
Ca2+ Homeostasis
Activation of Ca2+ Dependent Systems (Endonucleases, Proteases, & Phospholipases)
Hydroperoxides, Aldehydes etc.
Depletion of ATP and NADPH
Damage Repaired
Cell Survival
Xanthine Dehydrogenase
Xanthine Oxidase
Xanthine and Uric acid
Necrosis
Apoptosis
Cell Destruction

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(For Internal DTIC Use)

Title: COMMON PATHWAYS IN THE TOXICITY OF - - -

PoC: WALTER KOZUMBO Date: 10-13-99

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